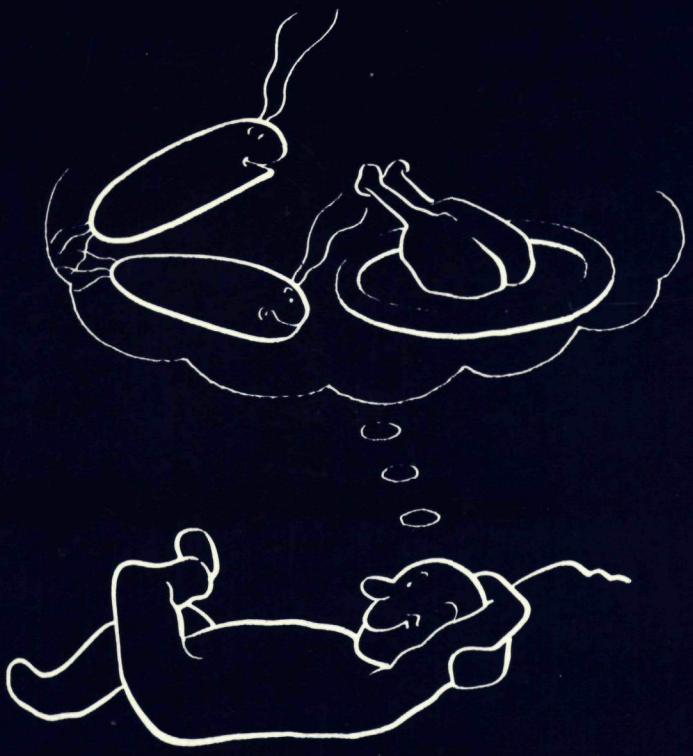
CHEMOTAXIS OF *Bacillus subtilis*



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CHEMOTAXIS OF BACILLUS SUBTILIS

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE WISKUNDE EN NATUURWETENSCHAPPEN AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN, OP GEZAG VAN DE RECTOR MAGNIFICUS PROF DR A J H VENDRIK VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN HET OPENBAAR TE VERDEDIGEN OP VRIJDAG 10 MAART 1978 DES NAMIDDAGS OM 2 UUR PRECIES

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C O N T E N T S

CHAPTER 1

GENERAL INTRODUCTION

History. Antonie van Leeuwenhoek was the first to report the movement of bacteria in his letter to the Royal Society of London on the 9th of October 1676 (Brock, 1975). However, it took more than two centuries before a systematic study of the chemotaxis (movement towards or away from chemicals) of bacteria was begun by Engelmann (1881) in his laboratory at Utrecht. He studied the accumulation of *Bacterium termo* in regions of high oxygen tension caused by cells of higher and lower plants undergoing photosynthesis. Later on Engelmann (1883) published a paper on another kind of tactic movement, the phototaxis of a purple sulfur bacterium, resembling *Chromatium okenii*.

In the next few years Pfeffer (1884) further extended the studies on chemotaxis by inserting into a suspension of bacteria capillaries filled with solutions of various compounds. He discovered that the bacteria reacted upon the gradient formed by diffusion of the compound out of the capillary tube; some of the compounds acted as attractants (positive chemotaxis), some as repellents (negative chemotaxis) and others were inert. Attractants and repellents were antagonistic and could partially or completely cancel each others effects, dependent on the respective concentrations (Pfeffer, 1888). Accumulation in the capillary occurred also, when attractant was present both

in the capillary tube and the bacterial suspension, provided that the concentration of attractant in the tube was higher by a fixed factor (Pfeffer, 1888).

Modern Studies A. Receptors. Adler (1969) quantified the capillary method of Pfeffer by counting the number of bacteria in the capillaries and used this method to prove that bacteria have specific chemoreceptors. He showed that (1) chemicals that are extensively metabolized are not necessarily attractants; (2) chemicals that are not metabolized can be attractants; (3) transport of chemical into the cell is neither necessary nor sufficient to provoke a chemotactic response; (4) structurally related compounds are competitors for the response toward an attractant; (5) mutants exsist which lack taxes to certain compounds but not to others. Both chemoreceptors for attractants (amino acids and sugars) and repellents are now characterized in *Escherichia coli* (Adler, 1975; Tso and Adler, 1974), *Salmonella typhimurium* (Tsang et al., 1973; Strange and Koshland, 1976) and *Bacillus subtilis* (de Jong et al., 1975; Ordal et al., 1977). Wherever these chemoreceptors have been identified, they were also shown to function in a transport system, for which the attractant of the chemoreceptor serves as substrate. Yet both the transport and chemotaxis systems have other independent components and transport is not required for chemotaxis.

The relationship between transport and chemotaxis is best illustrated with the galactose receptor of *E. coli* (Hazelbauer and Adler, 1971). The galactose binding protein is both required

for galactose chemotaxis as receptor and for the β-methylgalactoside transport system.

Mutants which lack one of the transport specific components, show chemotactive activities varied from that of the wild type to nearly complete inhibition, showing the complex interaction between those components and the chemosensing machinery (Ordal and Adler, 1974). More than 25 genes, necessary for motility and chemotaxis, have been identified in *E. coli*. The most important groups of these are the fla genes (necessary for flagellar assembly), the mot genes (for motility) and the che genes (for chemotaxis) (Silverman and Simon, 1977 I; Hilmen et al., 1974). The che mutants, usually called generally non-chemotactic mutants, are motile but do not show any chemotaxis at all (Armstrong et al., 1967). Other mutants (specific non-chemotactic mutants) are known which show, in the case of the galactose receptor, no chemotaxis towards galactose and the other compounds bound by the galactose binding protein or no chemotaxis towards galactose and ribose jointly (Ordal and Adler, 1974; Strange and Koshland, 1976).

Strange and Koshland (1976) showed that the joint inhibition of taxis towards galactose and ribose was due to competition for a common site in the chemotactic signalling system. Also in *B. subtilis* several receptors might compete for a common signalling site and so mutually influence each others response (de Jong et al., 1975).

Mutants which lack the galactose binding protein activity

are defective both in transport and in chemotaxis (Hazelbauer and Adler, 1971). But mutants with an altered galactose binding protein can show two other phenotypes (Adler, 1975): one that binds and transports galactose normally but fails to carry out chemotaxis (Ordal and Adler, 1974) and another with normal chemotaxis but with altered transport (Ordal and Adler, 1974). Thus the binding protein appears to have three sites, one for binding the ligand, one for interacting with the next transport component(s) and one for interacting with the next chemotaxis component(s).

The first step in the chemosensing system, the action of the receptors is relatively well known; so is the last step, the way in which the flagella control the motile behaviour of the bacteria. How the signal is transmitted from the receptors to the flagella is much less well known. The role of methionine in sensory adaptation has been established beyond doubt, down to the molecular level (Silverman and Simon, 1977 II). It is also known that a hyperpolarizing wave is originated in the transmittance of the chemotactic signal (Szmelcman and Adler, 1976). The way in which these components interact with each other and how the other components of the signalling system are involved remains speculative however (de Jong and van der Drift, 1978).

B. The flagellar motor. Recently it has been shown that the flagellar motor is driven by a proton flux (Manson et al., 1977). The motor can work both clockwise and counterclockwise

(Silverman and Simon, 1974). The flagella themselves are left-handed, semirigid helices, which form, in peritrichously flagellated bacteria, a coordinated bundle during counterclockwise rotation (MacNab, 1977). When the direction of rotation reverses, the bundle jams and disperses actively (MacNab, 1977).

Motile cells alternate between periods of swimming (counterclockwise rotation of flagella, i.e. smooth translational movement, runs) and brief randomizing events (clockwise rotation of flagella, i.e. twiddles) (Berg and Brown, 1972). In this way they achieve a random walk, composed of runs, interrupted by twiddles, that bring about a change of direction of translational movement. By regulating the twiddle frequency (number of twiddles / s) the bacteria are able to determine their general direction of movement. In isotropic solutions the twiddle frequency is fairly constant (about 0.15 twiddles / s for *B. subtilis*). When the bacteria swim up a gradient of attractant the twiddle frequency is being suppressed. When they swim with a gradient of attractant, the twiddle frequency is nearly the same as in isotropic solutions (Berg, 1975). So the bacteria drift up the gradient because the runs are somewhat longer in the favorable direction. In gradients of repellents the reverse holds. A direct proof that the mode of behaviour of the bacteria is controlled by the direction of rotation of the flagellar motor was given by Larsen et al. (1974), who tethered *E. coli* to a glass surface by means of their flagella and then applied temporal gradients of attractants. Increases in attractant con-

centration caused counterclockwise rotation of the flagella, increases in repellent concentration clockwise rotation.

C. Transmittance of the signal from the receptors to the flagella. It has rather long been known that methionine is required for transmission of the chemotactic signal (Armstrong, 1972 II). Furthermore the membrane potential was supposed to play a role too (Berg, 1975). Recently, Szmelcman and Adler (1976) showed that addition of attractant or repellent to *E. coli* cells brought about a hyperpolarizing wave, which was specific for the chemotactic mechanism. Remarkably both attractant and repellent caused a hyperpolarization, while attractants caused smooth swimming and repellents twiddles. So the membrane potential *per se* does not determine the twiddle frequency but other processes must be involved too (Szmelcman and Adler, 1976). De Jong et al. (1976) showed earlier that an artificial increase in the proton motive force can cause either twiddles or smooth swimming, but a decrease always caused twiddles. Simultaneous addition of attractant and application of an increase in proton motive force prolonges the smooth response towards the attractant. While simultaneous addition of attractant and application of a decrease in the proton motive force lessen the smooth response towards the attractant or even cause twiddles.

A number of paralyzed mutants, caused by an altered mot-gene product, surprisingly show no hyperpolarization wave after addition of attractant. Szmelcman and Adler (1976) suggest

that this mot-gene product is an ion-gate, which by its interaction with the receptors produces changes in ion-fluxes and hence in membrane potential.

The role of methionine in bacterial chemotaxis was first shown by Adler and Dahl (1967), who demonstrated that methionine starvation in *E. coli* resulted in a loss of chemotactic activity. Since then fairly detailed biochemical information about the role of methionine in chemotaxis has become available. First it was shown that not methionine itself but its metabolite S-adenosylmethionine is involved in chemotaxis (Armstrong, 1972 I and II; Aswad and Koshland, 1975). S-adenosylmethionine methylates a number of membrane bound proteins involved in bacterial chemotaxis. A specific methyl-transferase, which was shown to be the product of the che R gene in *S. Typhimurium*, played a role in this methylation (Springer and Koshland, 1977). Products of the che W and che X genes may play a similar role in *E. coli* (Silverman and Simon, 1977 II). Up till now products of the che D, che M and che Z genes of *E. coli* have been shown to become methylated. About the role of the product of the che Z gene, a cytoplasmatic protein, nothing is known in detail. The function of the products of che D and che M, the methylaccepting chemotaxis proteins (MCP's), is much better understood (Silverman and Simon, 1977 II; Springer et al., 1977 II). Adaptation to increased levels of attractant (or repellent) probably occurs by a slow methylation of the MCP's (Springer et al., 1977 I and II). Removal of the attractant (or repellent) results

in a much faster deadaptation, which may be provoked by a demethylation (Springer et al., 1977 I).

In *E. coli* it has been shown that the methylation of the che D product, MCP 1, is mostly stimulated by attractants from the serine taxis group and that methylation of the che M products MCP's 2 is mostly stimulated by attractants from the aspartate taxis group (Silverman and Simon, 1977 II; Springer et al., 1977 II).

However there is some overlap between both receptor groups. Besides Silverman and Simon (1977 II) demonstrated that bacteria with a defect MCP 1 showed no initial response to serine, whereas bacteria with defect MCP's 2 did not respond to 2-methylaspartate. So besides their function in adaptation / deadaptation to altered levels of attractant or repellent, the MCP's seem to be necessary for the processing of the initial chemotactic signal from the receptors to the flagella.

Moreover, methionine is required for the change in membrane potential caused by addition of attractants or repellents (Szmelcman and Adler, 1976). Evidence exists that the methylation site involved in this proces, probably located on the ion gates, differs from the methylation sites on the MCP's.

A number of models have been described for the mechanism of chemotaxis in bacteria. They will be discussed in detail in Chapter 7 of this thesis. Other models describe parts of the chemotactive machinery. Perhaps the most

interesting of these are the model for the action of the flagellar motor proposed by Berg (1974), and a model for the behaviour of flagellar bundles given by MacNab (1977).

Scope of the present investigation. *B. subtilis* can be considered to be one of the best studied gram-positive, motile, bacteria. It seemed attractive to study the chemotactic behaviour of *B. subtilis* and compare it with the chemotactic behaviour of the gram-negative *E. coli*. The aim of the study was to gain information on the chemorecepting machinery and the role of the proton motive force in the mechanism of chemotaxis. The study was restricted to the positive chemotaxis toward amino acids because transport of amino acids had been already studied extensively in *B. subtilis* (Konings and Freese, 1972). Two different techniques were used to study chemotaxis. (1) A quantitative capillary assay as first introduced by Adler (1969) and (2) direct microscopic observation of the cells after application of temporal gradients. The latter technique is much faster and can also be used to study the change in behaviour of cells upon addition of reagents which change the proton motive force. However, it can not be used to study weak responses as can be done with the capillary method.

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CHAPTER 7

GENERAL DISCUSSION

Introduction. This thesis deals with the chemotaxis of *Bacillus subtilis* studied essentially from a physiological point of view. The results obtained using this approach appear to be in accordance with the general picture emerging from physiological, biochemical and genetic studies of the chemotactic behaviour of *Escherichia coli* and *Salmonella typhimurium* (Adler, 1975). If the mechanism of chemotaxis is similar for *B. subtilis* (de Jong and van der Drift, 1978), *E. coli* (Adler, 1975), *S. typhimurium* (Springer and Koshland, 1977) and the motile Streptococcus strain V4051 (Manson et al., 1977; van der Drift et al., 1975) - so far no indications are known which contradict this hypothesis - then the model for the mechanism of chemotaxis given in chapter 6 can be considered as a general model. This common model has the following features: (1) specific receptors (Adler, 1975; de Jong et al., 1975); (2) a hyperpolarizing wave involved in the transmittance of the chemotactic signal (Szmelcman and Adler, 1976); (3) methylaccepting chemotaxis proteins (MCP's) for adaptation/deadaptation to changed levels of attractants or repellents (Springer et al., 1977 I and II); (4) runs and twiddles due to counterclockwise and clockwise rotation of the flagella respectively (Silverman and Simon, 1974).

However, one conspicuous point of difference exists between

our results and those obtained with gramnegative organisms. After methionine starvation cells of *E. coli* (Adler and Dahl, 1967) and *S. typhimurium* (Aswad and Koshland, 1975 II) swim continuously smoothly, but, in contrast to those of *B. subtilis* OI 151 (Ordal, 1976 I), cells of *B. subtilis* 60015, show a normal swimming behavior and no response toward attractants (de Jong et al., 1977). Although this different behavior can not be explained so far, we feel no reason to assume a major difference in the mechanism of chemotaxis between *B. subtilis* 60015 and the other bacteria studied so far.

Mutants. Some aspects of the mechanism of chemotaxis could not be studied in this thesis because of the lack of suitable mutant strains of *B. subtilis*. For example problems with classification of receptors (de Jong et al., 1975; Ordal et al., 1977) could have been solved if specific non-chemotactic mutants had been available. These mutants lack taxis to compounds detected by a single receptor, but responses mediated by other receptors are not impaired (Adler, 1975; Hazelbauer et al., 1969). Attempts to isolate those mutants were made using several methods. However, success was limited. In fact, a number of continuously twiddling or smoothly swimming (generally non-chemotactic) mutants and non-motile mutants were isolated, but not further characterized because of their limited usefulness in the physiological studies. Such mutants lack chemotactic behaviour (Silverman and Simon, 1977 I) and are isolated by lack of a

specific screening method for the specific non-chemotactic mutants in *B. subtilis*. The absence of motility in mutants can be due to the absence of flagella, or to the presence of incomplete flagella, e.g. polyhook mutants (Hilmen et al., 1974) or intact flagella which can not rotate (mot-gene mutants) (Armstrong and Adler, 1967). Besides the mutants mentioned above, tsr and tar mutants have been identified for *E. coli*. They lack taxis for a group of receptors due to altered MCP's (Springer et al., 1977 II; Silverman and Simon, 1977 II).

The methods used to isolate the mutants are generally based on the use of (1) swarm plates (soft agar plates), or (2) diffusing or preformed gradients of attractants (Aswad and Koshland, 1975 I), or (3) the isolation of transport-deficient mutants. The first method is based on repeated cycling of the cells which remain at the origin of a swarm plate (Armstrong et al., 1967). Moreover it can be used as a screening procedure for mutant colonies on the basis of their morphology in swarm agar (Ordal and Adler, 1974). Tso and Adler (1974) used the second method for the isolation of mutants for negative chemotaxis in *E. coli*. Many receptor mutants were first isolated as transport-deficient mutants (method 3) (Hazelbauer and Adler, 1971; Aksamit and Koshland, 1974; Parkinson, 1975), which indicated a relation between chemotaxis and transport (see introduction).

We used *B. subtilis* W_{23} in our studies of the isolation of non-chemotactic mutants. The chemotactic behavior of *B. subtilis* W_{23} strongly resembles that of *B. subtilis* 60015, but

B. subtilis W_{23} does not require L-methionine and L-tryptophan for growth. Mutations were induced by ultraviolet light and several mutagenic compounds. All three methods mentioned above (swarm plates, preformed gradients of attractants, and isolation of transport-deficient mutants) were applied to isolate specific non-chemotactic mutants of *B. subtilis*. From the enrichments about 5000 pure cultures were isolated and tested for their chemotactic behavior on swarm plates (0,18% agar). Although this method was successfully applied to *E. coli* to discern between non-motile, generally non-chemotactic, specific non-chemotactic and wild type strains (Parkinson, 1976; Ordal and Adler, 1974), with *B. subtilis* only non-motile strains could be discerned easily. The patterns on the swarm plates for the other kinds of mutants and the wild type strain were so complex, that even after a second screening with a number of selected strains, a final test with a capillary chemotaxis assay was necessary. Only one specific non-chemotactic strain was isolated, which showed a chemotactic response toward L-leucine of about 30% when compared to the wild type. It should be noticed here that also for *E. coli* all attempts to isolate receptor mutants for positive chemotaxis toward amino acids failed so far (Parkinson, 1975), although a number of mutants for taxis towards sugars and for negative taxis away from amino acids are known for this organism (Adler, 1975; Tso and Adler, 1974). The receptor mutants isolated so far for positive chemotaxis have mutations either in an osmotical shockable binding protein of the transport system

or in a phosphoenolpyruvate dependent phosphotransferase system (Adler, 1975). Part of the difficulties encountered in the isolation of receptor mutants for positive chemotaxis toward amino acids could have arisen because the amino acids are detected by more than one receptor (Parkinson, 1975).

Mechanism of chemotaxis. The first models proposed to explain the mechanism of taxis in bacteria were relatively simple. Links (1955) and Clayton (1953) proposed that bacteria reacted due to a sudden decrease in the rate of energy supply to the motor apparatus. In 1969 Adler proved that bacteria have specific chemoreceptors and therewith started a line of research which made clear that bacteria detect, process and respond to sensory information in a much more sophisticated manner. Specific knowledge has accumulated of various aspects of chemotaxis. These are, the receptors, the motion of individual cells, the motion of the flagella and the information processing from the receptors to the flagella. These aspects will be discussed in relation to the knowledge about the mechanism of chemotaxis of *B. subtilis* in particular as presented in this thesis. Finally various models resulting from integration of known data will be evaluated.

Receptors. In *B. subtilis* a number of receptors for taxis toward amino acids are known (de Jong et al., 1975; Ordal et al., 1977). Furthermore Ordal (1976 II) proposed that compounds like uncouplers of oxidative phosphorylation and local anesthetics, which

cause twiddles in *B. subtilis*, act at saturable and specific recognition sites. However, more likely these agents act by a general effect on the proton motive force thus affecting the behavior of the cells (de Jong et al., 1976; de Jong and van der Drift, 1978). Since Ordal did not measure Δ pH and $\Delta\psi$, simultaneously his conclusions about seperate binding sites on the membrane for different uncouplers are rather tentative. Moreover his view is in contrast with the current view on uncoupler action (Kessler et al., 1976, Kessler et al., 1977).

Motion of individual cells. The motion of individual cells in isotropic solutions, spatial and temporal gradients of attractants and repellents has been followed directly with the aid of a microscope, by tracks recorded photographically and in a number of cases with a tracking microscope (Vaituzis and Doetsch, 1969; de Jong et al., 1976; de Jong et al., 1977; Berg, 1971; Berg and Brown, 1972; Brown and Berg, 1974; MacNab and Koshland, 1972). In isotropic solutions the movement of the bacteria consists of random walks, runs, interposed with twiddles (Berg, 1975). In a positive, temporal or spatial, gradient of attractant, twiddles are suppressed. In a negative spatial gradient of attractant the twiddle frequency is the same as in isotropic solutions, whereas in a negative temporal gradient the twiddle frequency increases, although this effect is short lived. The difference found between spatial and temporal gradients might be accounted for by the difference in the steepness of the concentration gradients in both kinds of experiments

(Berg, 1975). Simultaneous addition of attractant and repellent caused an intermediate response, dependent upon the strength of the opposing stimuli (Tsang et al., 1973).

In essence all the observations reported on the motion of individual cells of *E. coli* and *S. typhimurium* have been made for *B. subtilis* too. In isotropic solutions the twiddle frequency of the cells is constant, although amino acids stimulate the velocity (de Jong et al., 1977). No observations of the behaviour of cells in spatial gradients of attractants or repellents are available. In increasing temporal gradients of attractants, the twiddle frequency strongly decreases, whereas in decreasing temporal gradients short periods of an increased twiddle frequency are observed (de Jong et al., 1976; de Jong et al., 1977). Addition of repellents causes an increase in the twiddle frequency (Ordal, 1976 II). Some of the repellents used also affected the proton motive force. Simultaneous addition of attractants and compounds, which cause a decrease in the proton motive force, caused an intermediate response. However, simultaneous addition of compounds which cause an increase in the proton motive force prolonged the response toward the attractant (de Jong et al., 1976; de Jong and van der Drift, 1978). Experiments in which simultaneously with the addition of attractants the proton motive force was manipulated have not been done for *E. coli* or *S. typhimurium*, but the results obtained with the motile *Streptococcus* strain V 4051 (Manson et al., 1977) appear to corroborate the results obtained with *B. subtilis*. Occasional observa-

tion of *B. subtilis* cells tethered to a glass surface revealed that these cells normally rotate counterclockwise, with occasionally a short period of clockwise rotation. Temporal gradients of attractants cause periods of continuous counterclockwise rotation (de Jong, unpublished results). Both observations have been made earlier for *E. coli* and *S. typhimurium* (Berg, 1975; Adler, 1975).

Motion of the flagella. Berg and Anderson (1973) have demonstrated that flagellar filaments rotate relatively to the cell body as rigid or semirigid helices. Later Silverman and Simon (1974) proved this very conclusively with polyhook and straight flagellar mutants which are non-motile when free, but rotate when tethered to a glass surface. The flagellar motor is driven by a proton flux (Manson et al., 1977) and runs remarkably smoothly (Berg, 1974). In this thesis it has been proposed that also in *B. subtilis* the flagellar motor is driven by a proton flux, though no definitive proof could be obtained (de Jong and van der Drift, 1978). Recently Matsura et al., (1977) induced, under somewhat different conditions, motility in starved *B. subtilis* cells by application of an artificial proton motive force.

Information processing from the receptors to the flagella. In *E. coli* and *S. typhimurium* methionine, via its metabolite S-adenosylmethionine is necessary to methylate the MCP's (Silverman and Simon, 1977 II; Springer and Koshland, 1977).

The role of the MCP's in sensory adaptation has been shown beyond doubt. It appears that the MCP's are also necessary for the transmission of the primary signal from the receptors to the other parts of the sensing system (Silverman and Simon, 1977 II).

Only recently it has been shown for *E. coli* that a hyperpolarizing peak in the membrane potential occurs, as part of the chemotactic response, both after addition of attractant or repellent (Szmelcman and Adler, 1976). When methionine was omitted no hyperpolarizing peak could be observed. Therefore Szmelcman and Adler (1976) proposed that a methylation/demethylation process controls the quantity or nature of ions that cause the hyperpolarizing peak. Since a mutant which fails to methylate the MCP's showed a normal hyperpolarizing peak they concluded that two methylation sites must be involved; one of the MCP's and another on an ion-gate controlling the twiddle frequency. Mot-gene mutants, which have intact but paralyzed flagella do not show a hyperpolarizing peak. Therefore Szmelcman and Adler (1976) proposed that the ion-gate with the second methylation site, might be the mot-gene product.

In *B. subtilis* 60015 the response toward attractant is repressed after methionine starvation (de Jong et al., 1977). A weak twiddle response is observed when cells starved in the presence of an attractant, were resuspended in a medium without attractant. This might be an indication that the presence of methionine is not required to retain a state of adaptation and that the twiddle response observed with a steep negative tem-

poral gradient of attractant, is a result of rapid deadaptation (de Jong et al., 1977; Springer et al., 1977 I). It was also shown that an artificial hyperpolarizing peak and simultaneous addition of attractant do not cause a smooth response in methionine-starved cells of *B. subtilis* (de Jong and van der Drift, 1978). This indicates that methionine is not only required for adaptation/deadaptation to changed levels of attractants or repellents, but also for the transmittance of the receptor signal to the flagella. So it may be assumed that in *B. subtilis* methionine plays a similar role as in *E. coli*, though no biochemical proof is available. A biochemical study of the role of methionine in the chemotaxis of *B. subtilis* would be an useful extension of the experiments described in this thesis.

The role of the proton motive force in the chemotactic behavior of *B. subtilis* is described extensively in this thesis. Quantitative relationships were found between the duration of the twiddle response and decreases in the Δ pH and $\Delta\psi$ caused by nigericin and valinomycin, respectively (de Jong and van der Drift, 1978). Although a sudden decrease in the proton motive force always causes twiddles, a sudden increase in the proton motive force can cause twiddles, a smooth or an intermediate response (de Jong et al., 1976). Simultaneous addition of attractants revealed that the response toward the attractant is prolonged by an increase in the proton motive force, but diminished by a decrease in the proton motive force.

So it appears that binding of an attractant, a hyperpolarizing peak and methionine are all necessary for a smooth response (de Jong and van der Drift, 1978). The duration of the response depends on the magnitude of the hyperpolarizing peak and probably the rate of methylation of the MCP's. In *E. coli* such a quantitative relationship between response and the magnitude of the ion fluxes has not yet been shown, though direct measurements of the hyperpolarizing peak after addition of attractants or repellents were made (Szmelcman and Adler, 1976).

Models. One of the first models in which the known data on the mechanism of chemotaxis were integrated was given by Adler (1975), but in this model the biochemical evidence for the role of methionine, which since then became available, was not included. Springer et al. (1977 II) found biochemical evidence for the role of methionine. They extended the model of Adler and emphasized the role of the MCP's in the process. They pointed out that nothing is known about the processing of the sensory information from the receptors to the MCP's and the flagella. Silverman and Simon (1977 II) proposed that the MCP's undergo a rapid change under influence of the receptor activity, which makes them available for methylation and induces the response (suppression of twiddles) possibly by a release of ions. Adaptation occurs by a slow methylation of the MCP's. Springer and Koshland (1977) proposed a model in which the rates of formation

and decomposition of a hypothetical twiddle generator X, whose concentration level controls the twiddle frequency, are emphasized. The receptors are capable of increasing the rate of both formation and decomposition of X. Methylation of the MCP's increases the rate of decomposition of X. In this model no distinct role is reserved for ion-fluxes, caused by addition of attractants or repellents, or for the influence of these fluxes on the functioning of the flagellar motor. Finally de Jong and van der Drift (1978) proposed a model in which a hyperpolarizing peak is originated by the receptors at a second methylation site, which may be a part of the flagellar motor. The MCP's are methylated under influence of the hyperpolarizing peak or the compensatory ion-fluxes following the peak.

All these models have a number of common features and as long as no detailed biochemical information about the interaction of the components of the sensory system is available, it can not be decided which model is the most accurate. It is obvious that a large number of experiments have to be designed and performed before this question can be answered unequivocally.

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Chemotaxis is the movement of living organisms towards or away from chemicals. In this thesis the chemotaxis towards amino acids of *Bacillus subtilis* was studied. Chapter 1 is a general introduction, which gives a historical view of the field and a short review of the modern literature. Special emphasis is put on receptors, the flagellar motor and the transmittance of the signal from the receptors to the flagella. In chapter 2 the quantitative responses of *B. subtilis* 60015 to commonly occurring L-amino acids were studied. Except for L-glutamate, L-aspartate, L-arginine and L-lysine all L-amino acids were found to be attractants. D-amino acids were no attractants. Responses were optimal between pH 6.0 and 7.0 and between 30° and 35°C. In chapter 3 the receptors for chemotaxis toward amino acids, were classified with the use of taxis competition experiments. Three receptors, the asparagine, the alanine and the isoleucine receptor could be identified. The isoleucine receptor possibly is a cluster of several receptors interfering with each other. L-histidine and L-glycine could not be assigned to these receptors. Possibly they are detected by a separate receptor or slightly by several others. In chapter 4 the effect of amino acids on the motile behavior of *B. subtilis* was studied. Constant levels of amino acids enhanced the velocity of the cells about 2-fold. The stimulation of velocity did not occur via the receptors for chemotaxis. Methionine starvation did not result in

constantly smoothly swimming cells, as is the case for *Escherichia coli* and *Salmonella typhimurium*. The *B. subtilis* cells kept swimming with a normal twiddle frequency, but were unable to react on temporal gradients of attractants. Remarkably, high concentrations of L-methionine and also of L-cysteine caused a similar effect. In chapters 5 and 6 the influence of the proton motive force on the chemotactic behavior of *B. subtilis* was studied. A quantitative relationship was established between the ion flux during a decrease of the proton motive force and the duration of the twiddle response. It was also shown that an artificial increase in the proton motive force simultaneously with the addition of an attractant prolonged the response toward the attractant. In methionine-starved cells no response was observed after such a treatment. A general model for the mechanism of chemotaxis was proposed considering the results with *B. subtilis*, *E. coli* and *S. typhimurium*. Chapter 7 is a general discussion which reviews the state of knowledge about the mechanism of chemotaxis of *B. subtilis* as compared to *E. coli* and *S. typhimurium*. Finally a number of models described in the literature for the mechanism of chemotaxis were compared to the model described in Chapter 6.

SAMENVATTING

Chemotaxis is de beweging van levende organismen naar of vanaf chemische verbindingen. In dit proefschrift werd de chemotaxis van *Bacillus subtilis* naar aminozuren bestudeerd. Hoofdstuk 1 is een algemene inleiding, met een historisch overzicht en een korte weergave van de stand van zaken in de recente literatuur. Speciale aandacht werd hierbij besteed aan receptoren, de flagellaire motor en de overbrenging van het signaal van de receptoren naar de flagellaire motor. In hoofdstuk 2 worden de concentratie-respons curves voor de chemotaxis van *B. subtilis* 60015 naar alle normaal voorkomend L-aminozuren gegeven. Behalve L-glutamaat, L-aspartaat, L-arginine en L-lysine waren alle L-aminozuren attractanten. D-aminozuren waren geen attractanten. De respons was optimaal tussen pH 6.0 en 7.0 en tussen 30° en 35°C. In hoofdstuk 3 werden de receptoren voor de chemotaxis naar aminozuren geklassificeerd met behulp van competitie experimenten. Er werden drie receptoren gevonden: de asparagine, de alanine en de isoleucine receptor. De isoleucine receptor is mogelijkerwijs een cluster van verschillende met elkaar interferende receptoren. L-histidine en L-glycine konden niet aan één van deze receptoren worden toegewezen. Mogelijkerwijs hebben ze een eigen receptor, of worden ze in geringe mate door meerdere receptoren herkend. In hoofdstuk 4 werd de invloed van aminozuren op de beweging van *B. subtilis* onderzocht. In homogene oplossingen van aminozuren werd de snelheid van de

cellen met een factor 2 vergroot. Deze snelheidsvergroting vond niet plaats via de chemotaxis receptoren. Methionine hongering had niet tot gevolg dat de cellen, zonder richtingsveranderingen rechtdoor bleven zwemmen zoals bij *Escherichia coli* en *Salmonella typhimurium* het geval is. *B. subtilis* cellen handhaafden de normale frequentie van richtingsveranderingen maar waren niet meer in staat te reageren op het plotseling toedienen van een attractant. Opmerkelijk was dat hoge concentraties L-methionine en L-cysteine een soortgelijk effect veroorzaakten. In hoofdstuk 5 en 6 werd de invloed van de "proton motive force" op de chemotaxis van *B. subtilis* bestudeerd. Er werd een kwantitatief verband gevonden tussen de ion fluxen die optraden bij een artificieel geinduceerde vermindering van de "proton motive force" en de duur van de "duikel" respons. Een gelijktijdig met de toevoeging van een attractant geinduceerde toename van de "proton motive force" verlengde de respons naar de attractant. In methionine gehongerde cellen trad totaal geen respons op na een dergelijke behandeling. Er werd een algemeen model gegeven voor het mechanisme van de chemotaxis, waarin de resultaten die gevonden zijn van *B. subtilis*, *E. coli* en *S. typhimurium* verwerkt zijn. Hoofdstuk 7 is een algemene discussie, waarin de kennis omtrent de mechanismen van de chemotaxis bij *B. subtilis*, *E. coli* en *S. typhimurium* werden vergeleken. Tenslotte werden in dit hoofdstuk een aantal modellen beschreven in de literatuur die het mechanisme van de chemotaxis moeten verklaren vergeleken met het model uit hoofdstuk 6.

C U R R I C U L U M V I T A E

De schrijver van dit proefschrift werd op 9 september 1950 te Nijmegen geboren. Hij behaalde in 1967 het H.B.S.-B diploma aan het Nijmeegs Lyceum en begon in hetzelfde jaar met de studie scheikunde aan de Katholieke Universiteit te Nijmegen. In september 1970 werd het kandidaatsexamen (S2) behaald. De doctoraalstudie omvatte twee hoofdrichtingen - organische chemie en chemische microbiologie - benevens één tentamen uit het algemeen gedeelte. Het doctoraal examen werd op 25 juni 1973 afgelegd. Tijdens de doctoraalstudie werd met veel plezier geassisteerd op het 1e jaars praktikum algemene chemie en les gegeven op de diëtistencursus van de Schutse. Vanaf 1 juli 1973 is de schrijver als wetenschappelijk medewerker verbonden geweest aan het Laboratorium voor Microbiologie.

STELLINGEN

I

De conclusie van Ordal dat ontkoppelaars van de oxydatieve fosforylering, werkzaam zijn via specifieke bindingsplaatsen is onvoldoende gefundeerd.
Dit proefschrift.

II

Het is aannemelijk dat *Methanosarcina barkeri*, energie wint bij de omzetting van acetaat naar methaan, door gebruik te maken van een fosforylering, die aan een electronen transportketen gekoppeld is.

III

De hoeveelheid experimentele gegevens, die ten grondslag ligt aan de berekeningen van Koyama over de bijdrage van de methaanbacterien in de grond aan de methaancyclus in de atmosfeer, is te gering.
T. Koyama. J. Geophys. Res. **68**, 3971-3973 (1963)

IV

De resultaten die Cappenberg en Prins vonden bij hun studie over de omzetting van acetaat naar methaan in modder, worden beter verklaard door een oxydatie van acetaat, zoals deze optreed in b.v. *Desulfotomaculum acetoxidans*, aan te nemen, dan door oxydatie van CH_4 door sulfaatreducerende bacterien te veronderstellen.
Th.E. Cappenberg and R.A. Prins. Antonie van Leeuwenhoek **40**, 457-469 (1974)
F. Widdel and N. Pfennig. Arch. Microbiol. **112**, 119-122 (1977)

V

Toewijzing van een definitieve structuur, op grond van hun NMR spectra, aan de cyclobuteenderivaten, ontstaan door een gesensitiseerde fotoadditie van dimethyl acetyleendicarboxylaat met 2-methylbenzo[b]furaan, kan niet met volledige zekerheid gebeuren.
A.H.A. Tinnemans and D.C. Neckers. J. Org. Chem. **42**, 2374-2377 (1977)

VI

De studie van Kolodny et al., over plasmatestosterongehaltes bij de mens, geeft aanleiding tot het leggen van ongefundeerde causale verbanden.
R.C. Kolodny et al. New England J. Med. **285**, 1170-1174 (1971)
Handleiding Sexuologie 1976-1977 pag. 139-140 Uitgave Faculteit der Geneeskunde K.U.

VII

Het positieve effekt van de milieu-effektrapportage zal groter zijn als deze plaatsvindt binnen het kader van de bestaande wetgeving en besluitvorming, dan wanneer de milieu-effektrapportage overeenkomstig het Amerikaanse voorbeeld in Nederland zou worden ingevoerd.

VIII

De wetenschappelijke karrière van de man, belemmert de emancipatie van zijn vrouw.

IX

Het sluiten van de bisonbaai voor het publiek, zou de diversiteit van de rekreatiemogelijkheden rondom Nijmegen ernstig verminderen.

X

De meerderheid denkt te weten wat "normaal" heet, dankzij het "afwijkend" gedrag van de minderheid.

Nijmegen, 10 maart 1978 M.H. de Jong